

1 **DIETARY EFFECTS ON INSULIN AND GLUCAGON PLASMA LEVELS IN**  
2 **RAINBOW TROUT (*Oncorhynchus mykiss*) AND GILTHEAD SEABREAM**  
3 **(*Sparus aurata*)**

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6 <sup>1</sup>Rojas, P., <sup>1</sup>Albalat, A., <sup>1</sup>Santigosa, E., <sup>2</sup>Pérez-Sánchez, J., <sup>3</sup>Kaushik, S. J.

7 <sup>1</sup>Gutiérrez, J., <sup>1</sup>Navarro, I.

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10 <sup>1</sup>Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda.

11 Diagonal 645, Barcelona, Spain

12 <sup>2</sup>Instituto Acuicultura Torre de la Sal (CSIC), Castellón, Spain

13 <sup>3</sup>UMR Nutrition Aquaculture Génomique, INRA, Saint Pée-sur-Nivelle, France

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22 Please address all correspondence to I. Navarro

23 Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda.

24 Diagonal 645, Barcelona, Spain E-0807.

25 Fax: 34+ 93+ 4110358

26 E-mail: [mnavarro@ub.edu](mailto:mnavarro@ub.edu)

27

1 **Abstract**

2           The effects of dietary amino acid profile (based on muscle (M) or whole body  
3 composition (WB) and the balance between indispensable (IAA) and dispensable amino  
4 acids (DAA) in the diet on plasma levels of insulin and glucagon were analyzed in  
5 rainbow trout and gilthead sea bream.

6           Plasma insulin values (baseline and 6 h post-feeding) were higher in trout than in  
7 sea bream, but the relative post-feeding increase was more pronounced in sea bream.  
8 Within the same dietary amino acid profile, diets with lower IAA/DAA, had a lower  
9 effect on the post-feeding secretion of insulin in both species. Circulating levels of  
10 glucagon (baseline and post-feeding relative increases) were higher in sea bream. In  
11 trout, diets with WB amino acid profile had a greater secretory effect on post-feeding  
12 glucagon than did diets with M profile, while gilthead sea bream showed an inverse  
13 response to circulating glucagon with respect to diet. Muscle insulin and insulin growth  
14 factor-I binding parameters were not affected by the dietary regimen.

15           The postfeeding glucagon response depends on both the dietary AA profile and  
16 the fish species, while that of insulin seems to be more uniform, and is affected in a  
17 similar way regardless of the species.

18

## 1 **1. Introduction**

2 Partial replacement of dietary fish meal protein with plant protein has been  
3 successfully accomplished in a number of teleostean fishes (Burel et al., 2000; Kaushik  
4 et al. 1995; 2004; Watanabe et al. 1998). However, fish meal and plant protein differ in  
5 a number of ways, including protein and energy content, amino acid profile and mineral  
6 composition. Another limitation of the use of vegetable compounds is that plant  
7 ingredients contain a certain proportion of anti-nutritional factors (Francis et al. 2001).  
8 Furthermore, with respect to indispensable amino acids (IAA) requirements of animals,  
9 plant proteins are often limited in one or more amino acids (Sauvant et al. 2004), and  
10 therefore in some cases, diets have to be supplemented with amino acids to avoid  
11 deficiencies and maintain adequate amino acid profile for correct growth.

12 The pancreatic hormones, insulin and glucagon, are known to play a key role in  
13 regulating the uptake of nutrients by tissues during the postprandial period (Navarro et  
14 al., 1993). Some studies reveal that levels of these hormones increase a few hours after  
15 food ingestion and contribute to post-feeding amino acid and glucose clearance (Sundby  
16 et al., 1991; Navarro et al., 1993).

17 Insulin is an anabolic hormone that stimulates the uptake of nutrients and  
18 incorporation by tissues (Duncan et al., 1998; Pérez-Sánchez and Le Bail, 1999; Peter  
19 and Marchant, 1995). In mammals, glucose is the main insulin secretagogue, along with  
20 some amino acids, whereas in fish, amino acids are more potent than glucose in  
21 stimulating insulin release (Mommsen and Plisetskaya, 1991). However, the response of  
22 insulin to dietary protein source (vegetable versus fish meal) and specially to the dietary  
23 amino acid profile has been poorly studied. Only the insulinotropic activities of various  
24 injected amino acids has been compared in the flounder (Andoh, 2007). Insulin and  
25 other peptides of its family, such as insulin growth factor-I (IGF-I), act through specific

1 tyrosine kinase membrane receptors (Le Roith et al., 1995). In fish, IGF-I is not only a  
2 growth factor, but also acts as a metabolic regulator both *in vivo* and *in vitro* (Castillo et  
3 al., 2004; Wood et al., 2005).

4 In mammals, glucagon also increases after a rich protein meal and it has been  
5 considered a pivotal hormone in amino acid disposal during an amino acid load. A  
6 pattern of biphasic increases in circulating post-feeding glucagon has been observed in  
7 both rainbow trout and European sea bass (Navarro et al., 2002), and the second  
8 glucagon peak has been suggested to be related to the increase in postprandial amino  
9 acids (Navarro et al., 2002). Furthermore, glucagon is known to enhance the uptake of  
10 amino acids in fish liver and stimulate the activities of aminotransferases (Inui and  
11 Ishioka, 1983). Information regarding the structure and function of fish glucagon and  
12 related peptides has increased in the last years (reviewed by Plisetskaya and Mommsen,  
13 1996; Moon, 1998; Mommsen, 2000; Mommsen and Busby, 2006). Numerous *in vitro*  
14 studies revealed that glucagon activates hepatic gluconeogenic pathway and glucose  
15 output. However, the role of glucagon in relation to dietary factors still remains poorly  
16 studied.

17 The present studies were part of a multidisciplinary project on the effects of diets  
18 with plant protein and with different dietary amino acid profiles on metabolism and the  
19 somatotropic axis in sea bream (Gómez-Requeni et al., 2003) and trout. Here we  
20 analyze the response of pancreatic hormones, insulin and glucagon, after adaptation to  
21 diets with low content of plant protein and different amino acid profiles and  
22 indispensable/dispensable amino acid ratios (IAA/DAA ratio) in relation to nutrient  
23 utilization and growth in gilthead sea bream and rainbow trout.

24

## 25 **2. Material and methods**

## 1 2.1. *Experimental diets*

2 Four experimental diets based on fish meal and plant ingredients (33-35%  
3 replacement) supplemented with free amino acids were developed for rainbow trout (T)  
4 and gilthead sea bream (SB) (Tables 1 and 3). For each species, two of the diets (M and  
5 WB) were based on the IAA profile and DAA content of muscle (M) and whole body  
6 (WB), respectively. In M2 and WB2 diets, DAA content and the IAA/DAA ratio was  
7 changed through the incorporation of glutamic acid. For dietary amino acid composition  
8 of diets for sea bream, see reference Gómez-Requeni et al., 2003. Dietary amino acid  
9 composition of diets for trout is shown in table 2.

10

## 11 2.2. *Animals and growth trials*

12 Growth trials with rainbow trout (*Oncorhynchus mykiss*) were conducted in the  
13 INRA experimental fish farm (Donzacq, France) at a constant water temperature of  
14  $17 \pm 1$  °C. Studies with gilthead sea bream (*Sparus aurata*) were carried out at the  
15 Instituto de Acuicultura, CSIC, de Torre la Sal (Castellón, Spain), where water  
16 temperature ranged naturally from 17 to 25 °C. For both species, each experimental diet  
17 was hand distributed to triplicate groups of fish for each diet in tanks of 500-l capacity,  
18 twice a day (9 h and 16 h) to near satiation (visual observation of the first refusal of  
19 feed); the quantity of food was recorded daily over the whole trial which lasted 12  
20 weeks.

21

### 22 2.2.1. *Postprandial experiment*

23 Blood samples were taken at the end of growth trials. Twelve fish from each  
24 treatment and for each sampling time (4 fish of each tank) were anaesthetised in 100  
25 ppm of 3-aminobenzoic acid ethyl ester (MS222) and blood was taken by caudal

1 puncture, 6 and 24 h after feeding. The two sampling times were done in two different  
2 consecutive days in different individuals. White lateral muscle was sampled 24 h after  
3 feeding and frozen in liquid nitrogen for insulin and IGF-I binding studies.

#### 4 5 *2.2.2. Force-feeding experiment*

6 After 12 weeks of adaptation to the diets, (17°C for rainbow trout; 21°C for  
7 seabream) we examined the response of plasma glucose, insulin and glucagon after  
8 force feeding. Fifty fish (either rainbow trout or gilthead sea bream) adapted to each of  
9 the TM1, TM2, SBM1 and SBM2 diets were divided into five groups of ten fish each  
10 and distributed in tanks of 90-l capacity. After 48 h of fasting, fish were force fed with  
11 the respective diet by means of stomach intubation at a rate of 1% body weight. Blood  
12 samples were taken under anaesthesia with MS222 from a group of ten fish at one of the  
13 following time periods: 1, 3, 6, 12 and 24 h after force feeding.

#### 14 15 *2.5. Analytical methods*

16 Plasma was collected after blood centrifugation (3000 x g, 10 min) and was split  
17 into three fractions: for glucose, glucagon and insulin analysis, respectively. A protease  
18 inhibitor, Trasylol (Bayer) was added to the plasma fractions (1000 IU/ml plasma) for  
19 glucagon analysis. All the plasma aliquots were kept frozen (-20°C).

20 Plasma glucose levels were analyzed by the glucose-oxidase colorimetric  
21 method (GLUCOFIX; Menarini Diagnostics, Firenze, Italy) (Huggett and Nixon, 1957;  
22 Sala-Rabanal et al., 2003). Insulin levels were measured by radioimmunoassay (RIA)  
23 using bonito (*Thunnus thynnus*) insulin as standard and a rabbit anti-bonito insulin as  
24 antiserum (Gutiérrez et al., 1984). It has been probed that this RIA is valid to measure  
25 trout and sea bream plasma samples and the antibody cross react with other fish species

1 studied (Navarro et al., 2002). Plasma glucagon levels were quantified by a  
2 heterologous radioimmunoassay method validated for fish plasma (Gutiérrez et al.,  
3 1984; Navarro et al., 1995).

4 Partial purification of solubilized insulin and IGF-I receptors from white muscle  
5 was performed at 4°C, as described by Párrizas et al. (1995) by affinity chromatography  
6 on wheat-germ agglutinin (WGA) bound to agarose (Vector Laboratories, Burlingame,  
7 USA). The glycoproteins obtained were measured following the method described by  
8 Bradford (1976). Binding assays were performed as in Párrizas et al. (1994). A volume  
9 of 30–40 µl of the WGA eluate (approximately 30 µg of glycoproteins) was incubated  
10 for 14–16 h at 4 °C with increasing concentrations of unlabelled hormone (from 0 to  
11 100 nM, final dilution) and radio-labelled ligand as tracer (25 pM, 50µCi/l). Semi-  
12 purified receptors were precipitated by addition of 0.08% bovine  $\gamma$ -globulin and 10.4%  
13 polyethylene glycol (final concentrations), followed by centrifugation at 14,000×g for 7  
14 min at 4 °C. Binding data were analysed in Scatchard plots and only the high-affinity,  
15 low-capacity binding sites were considered in the analysis. Porcine insulin was obtained  
16 from Lilly (Indianapolis, USA) and human recombinant IGF-I from Chiron  
17 (Emeryville, CA, USA). Human Tyr A14 <sup>125</sup>I-monoiodoinsulin and human recombinant  
18 3-<sup>125</sup>I-IGF-I, both with 2000 Ci/mmol specific activity, were purchased from Amersham  
19 Life Sci. (Arlington Heights, IL). All other chemicals used were purchased from Sigma  
20 (St. Louis, MO, USA).

21

## 22 2.6. Statistical analysis

23 Values are given as means with standard errors. Statistical analysis was  
24 performed using SPSS 11.5.1. The effect of the dietary adaptation was analysed for the  
25 different parameters using the Student's *t* test and Student Newman-Keuls test ( $p < 0.05$ ).

1 Data from force-feeding experiments were subjected to ANOVA and means were  
2 compared using the HSD Tukey test or Games-Howell test ( $p < 0, 05$ ).

3

### 4 **3. Results**

#### 5 *3.1. Growth performance and nutritional parameters in rainbow trout and sea bream*

6 Data on growth performance in juvenile rainbow trout are shown in Table 4.  
7 Final body weight and growth rates did not vary between the groups. Similarly, in  
8 juvenile gilthead sea bream, there was no effect of diet on specific growth rates and  
9 FGR of SBWB2 group was higher (Table 5).

10 Trout fed TM2 (based on muscle profile and supplemented with Glu) showed a  
11 lower hepatosomatic (HSI) index, as well as a lower feed gain ratio (FGR) and protein  
12 efficiency (PER).

13 Similarly, in sea bream, the lowest value for HSI was found in those fed the diet  
14 SBM2. In fact, HSI values from sea bream fed diets with the muscle IAA profile were  
15 lower than those fed the diet reflecting the whole body AA profile. No differences were  
16 observed between diets in terms of the protein efficiency ratio, and feed gain ratio  
17 values were similar across the different groups.

18

#### 19 *3.2. Plasma glucose, insulin and glucagon levels after growth trial in rainbow trout and* 20 *gilthead sea bream*

21 In trout, plasma glucose levels 6 h after feeding were higher than baseline  
22 plasma values at 24 h (Figure 1A) in all groups. Glycaemia was not different between  
23 diets at either of the two sampling times. In gilthead sea bream, plasma glucose values  
24 showed some variations between experimental groups only 6 hours after feeding (Figure  
25 1B). Baseline and post-feeding glucose values were lower than in rainbow trout.



1 Plasma insulin levels in trout at 24 h were similar among the different groups  
2 (range from 19,3±2,3 to 21,4±1,5 ng/mL). Insulin levels increased 6 h after feeding to  
3 approximately 30% above baseline values (range 27,5±1,5 to 31,9±0,7 ng/mL.  
4 Postprandial insulin levels from TM2 (lower IAA/DAA ratio) was lower than those fed  
5 TM1 (Figure 1C). Baseline plasma insulin levels (24 h) in gilthead sea bream were not  
6 different between diets, with values from 5,6±0,3 to 6,2±0,6 ng/mL. The relative post-  
7 feeding increase in insulin levels was higher than in trout, with plasma insulin values at  
8 6 h being approximately twice those observed at 24 h after feeding (Figure 1D). Insulin  
9 levels 6 h after feeding in SBM2 and SBWB2 were lower than in SBM1 and SBWB1  
10 groups, respectively.

11 Circulating glucagon levels in trout at the end of the growth trial (at 24 h post-  
12 feeding) were similar between groups, ranging from 0,6±0,1 to 0,7±0,1 ng/mL.  
13 Glucagon levels were higher 6 h after feeding (in relation to baseline values) only in  
14 TWB and TWB2 groups (Figure 1E). Circulating glucagon levels of sea bream at 24 h  
15 were higher than in trout (between 2,1±0,4 and 2,6±0,4 ng/mL). The relative increase in  
16 hormone levels after 6 h was also more pronounced than in trout, with glucagon levels  
17 three-fold higher than baseline values. Postprandial plasma glucagon levels in the  
18 different sea bream groups showed an inverse profile compared to that in rainbow trout.  
19 Seabream fed diets WB and WB2 had slightly lower values than those of M groups, the  
20 maximum values being observed in the SBM1 group (Figure 1F).

21

### 22 *3.3. Characterization of muscle insulin and IGF-I receptors*

23 Insulin and IGF-I binding parameters from muscle preparations of trout and sea  
24 bream 24 h after feeding are shown in Tables 4 and 5. In both species, IGF-I specific  
25 binding was higher than that of insulin, but in sea bream the difference was very

1 pronounced (three fold). Neither affinity (Kd) nor number of insulin or IGF receptors  
2 were affected by diet in sea bream. In trout, the number of IGF-I receptors changed  
3 between groups been higher in TM1..

#### 5 *3.4. Force-feeding experiments in rainbow trout and gilthead sea bream*

6 In both species, plasma glucose levels reached maximum levels 1 h after  
7 force feeding in trout (TM1  $13,4 \pm 1,2$  and TM2  $13,3 \pm 0,7$  mM) and sea bream (SBM1  
8  $13,2 \pm 0,6$  and SBM2  $10,7 \pm 0,5$  mM). Glycaemia decreased progressively across the post-  
9 feeding period, but fell more rapidly in gilthead sea bream (Figures 2A and 2B).

10 In rainbow trout, circulating insulin showed a similar pattern in both groups  
11 during the postprandial period, with increasing values at 3 h and maximum levels at 6 h  
12 after feeding. However, hormone levels in the TM1 group were higher than in TM2, at  
13 12 and 24 h after food administration (Figure 2C). Circulating glucagon levels in the  
14 TM1 group did not change over time (between  $1,4 \pm 0,2$  and  $1,6 \pm 0,1$  ng/mL), while in  
15 TM2 fish, levels reached their maximum at 1 h and decreased progressively to the  
16 minimum at 24 h. Hormone levels were similar between diets except at 24 h, when TM1  
17 levels were higher than TM2 values.

18 In gilthead sea bream, force feeding did not induce changes in plasma levels of  
19 pancreatic hormones. Plasma insulin and glucagon levels were not different between  
20 diets (Figures 2D and 2F).

## 22 **4. Discussion**

23 Great efforts have been made to reduce the level of fish meal in fish feeds by  
24 replacing fish meal with plant protein ingredients (Kaushik et al., 1995; Watanabe et al.  
25 1998; Vielma et al., 2000); the effects of fish meal replacement are not well known in

1 sea bream (Gómez-Requeni et al., 2004). Problems related to the presence of anti-  
2 nutritional factors or certain amino acid imbalances (Francis et al., 2001; Kaushik et al.,  
3 1995), are common to most species. Hormonal control of plant protein utilisation has  
4 been poorly studied and, as far as we know, there is no information on the dietary  
5 effects of the amino acid profile and IAA/DAA ratio on circulating insulin and glucagon  
6 in fish.

7         The results obtained in both species show that increasing the amount of DAA  
8 amino acids (glutamic acid) did not affect growth, irrespective of the IAA profile.  
9 However, growth was slightly impaired in groups fed diets with lower IAA/DAA. In  
10 rainbow trout, the negative impact of TM2 in FCR and PER appears not to be related to  
11 the DAA content because these effects would then have been found in the TWB2 diet  
12 group with the lowest IAA/DAA ratio, too. It is possible that this is related to the high  
13 soy bean meal level in diet TM2. However, it should be noted that the SBM2 diet, with  
14 similar composition, did not have such negative effects suggesting that trout are more  
15 sensitive to soybean meal than gilthead sea bream. Nevertheless, M2 diets did induce  
16 the lowest hepatosomatic indices in both species.

17         Diets with high carbohydrate content have been reported to induce a  
18 proportional increase in postprandial glucose in trout (Novoa et al., 2004). In the present  
19 study, the absence of notable changes in glycaemia between the groups is consistent  
20 with the composition of the diets, which were designed to present different amino acid  
21 profiles but with similar carbohydrate content. Furthermore, circulating insulin is not  
22 always well correlated with glycaemia levels and it appears to be more related to dietary  
23 protein level and composition (Navarro et al., 2002).

24         Baseline levels of insulin (24 h) were higher in trout than in sea bream, in line  
25 with previous reports (Navarro et al., 2002). These differences may be related to the

1 variations in the rates of basal hormone secretion or degradation. But no data on these  
2 possible differences between fish species are available. However, the postprandial  
3 increase relative to baseline values, irrespective of the dietary treatment, was much  
4 higher in sea bream, thus suggesting that the response of insulin to food ingestion is  
5 relatively higher in this species. The reasons for such differences are not known,  
6 because the only data regarding possible insulin secretagogues in sea bream indicate  
7 that administration of arginine induces lower increases in plasma insulin levels in sea  
8 bream than in salmonids (Vega-Rubin de Celis et al., 2004). Nevertheless, the  
9 sensitivity of insulin to many other factors (carbohydrates, gastrointestinal hormones or  
10 neural stimulus) might be enhanced in sea bream during the postprandial period.

11         The secretagogue effect of the different diets on circulating plasma insulin levels  
12 seems to present a similar pattern in both species. For a given dietary AA profile  
13 (muscle or whole body), groups fed with a higher IAA/DAA ratio exhibit higher plasma  
14 insulin levels 6 h after feeding. The presence of different proportions of some amino  
15 acid components of the different diets could exert a differential stimulation of insulin  
16 secretion. In fact, some amino acids such as arginine, lysine, leucine and phenylalanine  
17 increase insulin in fish both in vivo and in vitro (Ince and Thorpe, 1977; Matty and  
18 Lone, 1985; Navarro et al., 2002; Plisetskaya et al., 1991). Among these amino acids,  
19 arginine has been demonstrated to have a strong stimulatory effect on insulin secretion,  
20 especially in salmonids (Mommsen and Plisetskaya, 1991), although a dietary excess  
21 does not seem to induce such an insulintropic action in the Atlantic salmon (Lall et al.  
22 1994). In general, all these amino acids with an insulintropic action are considered  
23 essential, this being consistent with the higher insulin values observed in fish fed diets  
24 with higher IAA/DAA. Although in salmonids some studies suggest a correlation  
25 between circulating insulin levels and fish size (Plisetskaya, 1989; Sundby et al., 1991)

1 the role of insulin stimulating growth appears to be permissive. In mammals, normal  
2 portal insulin levels are required to maintain liver insulin growth factor-I (IGF-I)  
3 production and normal growth, and a post-feeding increase in plasma insulin is needed  
4 to ensure homeostasis. Insulin administration increased plasma IGF-I levels in trout  
5 (Baños et al, 1999), and both circulating insulin and IGF-I oscillate in parallel in  
6 response to some physiological situations (Baños et al., 1999; Larsen et al., 2001). The  
7 profile of differences in plasma IGF-I between dietary treatments, reported in sea bream  
8 fed the same diets (Gomez-Requeni et al., 2003), are similar to those found in insulin  
9 values in the present study, the minimum circulating IGF-I level being found in the  
10 WB2 group with the lowest growth. Thus, it appears that increased post-feeding insulin  
11 levels might contribute to a “good hormonal scenario” which permits an optimal  
12 nutritional and growth process. However, baseline insulin plasma levels did not exhibit  
13 changes associated with dietary adaptation, which is in agreement with the maintenance  
14 of insulin binding parameters. Indeed, only large changes in hormone levels can induce  
15 variations in the number of receptors available in the membrane (Navarro et al., 1999).

16 In contrast to insulin, baseline circulating levels of glucagon were lower in trout  
17 than in sea bream, and were in the range of those reported in previous studies (Gutiérrez  
18 et al., 1986; Navarro et al., 1991; Navarro et al., 1992). For many years the only  
19 reference for glucagon levels in sea bream has been the report by Gutiérrez et al. (1986).  
20 In that study, glucagon levels were analyzed in different species of fish and were found  
21 to be highest in sea bream, with levels 2- to 8-fold higher than those of other teleost  
22 species studied. More recently, it has been demonstrated that arginine increases  
23 circulating glucagon levels in sea bream even more than insulin (Navarro et al., 2002);  
24 this contrasts with what happens in salmonids, where the insulinotropic action often  
25 predominates over glucagon stimulation (Carneiro et al., 1993). However, no data are

1 available on the effects of other amino acids on glucagon secretion in fish, and even in  
2 mammals the information is limited and contradictory (Gannon et al., 2002; Nuttall et  
3 al., 2006). Nevertheless, the blood amino acid concentration after a protein meal is  
4 known to stimulate glucagon release in the dog (Pek et al., 1969), and the same is true  
5 for amino acid mixtures reproducing the physiological pattern in vitro. Nevertheless,  
6 differences exist between carnivorous and omnivorous mammals. Although some amino  
7 acids stimulate both insulin and glucagon secretion, it is also reported that the main  
8 gluconeogenic amino acids are among the most potent alpha cell stimulators, a feature  
9 which may be of physiological significance (Rocha et al., 1972). In fish, the dispensable  
10 amino acids alanine, aspartate and glutamate are potentially important as gluconeogenic  
11 substrates (Moon and Foster, 1995). In agreement with these observations the present  
12 study in trout showed that within a profile (muscle or whole body), groups fed a lower  
13 IAA/DAA ratio, with a higher proportion of some of those gluconeogenic amino acids,  
14 presented slightly higher glucagon levels. It is believed that in mammals the aminogenic  
15 glucagon release serves to stimulate hepatic glucose production and to avoid  
16 hypoglycaemia resulting from the concomitant insulin secretion (Nuttall et al., 2006).  
17 This has not been confirmed in fish, in which the control of glycaemia is not a relevant  
18 feature and where baseline levels of gluconeogenesis maintain a modest glucose  
19 turnover (Hemre et al., 2001). Nevertheless, glucagon does stimulate gluconeogenesis  
20 from amino acids in hepatocytes of eel and trout (Inui and Ishioka, 1983; Inui and  
21 Yokote, 1977), and post-feeding circulating glucagon is inhibited by dietary glucose  
22 (Novoa et al., 2004). In contrast, it has been described in trout that partial substitution of  
23 dietary protein by a single gluconeogenic dispensable amino acid can lead to an  
24 inhibition of gluconeogenic liver enzymes, but interestingly, without affecting  
25 glycaemia levels (Kirchner et al., 2003).

1           A different profile of glucagon secretion was found between diets in sea bream,  
2 and the pattern of secretion was more similar to that observed for insulin. Differences  
3 between species in the sensitivity of glucagon to various amino acids cannot be ruled  
4 out as a possible explanation. In this regard, the secretion of glucagon in sea bream  
5 appears to be especially sensitive to the essential amino acid arginine (Vega-Rubín de  
6 Celis et al., 2004). Furthermore, and in contrast to mammals, parallel changes in  
7 circulating insulin and glucagon values are commonly found in fish (Mommsen and  
8 Plisetskaya, 1991). The post-feeding increase in glucagon irrespective of the diet  
9 administered was also higher in sea bream than in trout. In mammals, a balanced  
10 response between insulin and glucagon after a protein-rich meal is needed for an  
11 optimal utilisation of nutrients. However, we cannot deduce from these experiments that  
12 the specific response of pancreatic hormones contributes directly to a better adaptation  
13 to diets in sea bream.

14           The force-feeding experiments enabled us to be sure that all animals sampled  
15 had eaten the same ratio of food and at the same time, thus eliminating variability in the  
16 hormonal response. In this way, these experiments permit us to check the effects of the  
17 same levels of ingested food for the different diets. In addition, they were designed to  
18 provide a complete hormone profile across the post-feeding period. In force-fed trout,  
19 the insulin profile across the various sampling times was similar with both diets, but  
20 with higher levels in the TM1 diet, this being consistent with the data from normal  
21 administration of feed. This suggests that differences between diets as regards insulin  
22 levels 6 h after normal feeding are really due to the intrinsic insulintropic capacity of  
23 dietary components, rather than being a possible consequence of differences in intestinal  
24 transit rate or quantity of ingested food, parameters which are controlled and are the  
25 same for both diets in forced feed experiment. Nevertheless, the observed profile with

1 maximum levels 6 h after feeding differs from that described previously for trout during  
2 standard feeding administration experiments, where very high insulin levels were  
3 already reached 1-2 h after ingestion of food (Pérez et al., 1988; Navarro et al., 1993;  
4 Novoa et al., 2004). High levels of insulin are actually found even earlier, around the  
5 time of food administration, when a reflex pre-absorptive release of insulin is produced;  
6 this is related to the visual appeal of food, the time feeding schedule, progressive  
7 stomach distension and other stimulating factors (Papatryphon et al., 2001). It appears  
8 that with the method of force feeding, which avoids all these external stimulating  
9 factors, the early secretion of insulin is reduced. However, force feeding would appear  
10 not to be the best method to study glucagon response in trout. Plasma glucose and  
11 glucagon levels were higher than in normal feeding conditions and remained high  
12 throughout the experimental period, thus suggesting a stress effect due to fish handling.  
13 Indeed, glucagon levels have been previously related to stress conditions, such as  
14 netting in tanks or handling (Navarro et al., 1992). It is also quite possible that under  
15 force-fed conditions, dietary nutrient supply is perhaps higher than that under voluntary  
16 feeding conditions.

17 In sea bream, force feeding did not induce changes in plasma levels of  
18 pancreatic hormones. In this species, the cephalic phase may be even more necessary  
19 than in trout, considering that the increase in insulin after force feeding was much more  
20 evident in trout than in sea bream. The fact that no differences were found in insulin  
21 levels between diets could suggest that maybe other factors than the composition of  
22 diets are also affecting the postprandial hormonal response during normal  
23 administration of food. Nevertheless, we cannot be sure because the insulin profile over  
24 time does not fit with the postprandial increases expected (Pérez et al., 1988). It is  
25 possible that the response to force feeding in this species may not represent the real



1 physiological situation. The levels of glucagon found, which were lower than in a  
2 normal feeding situation, suggest a different response of this hormone to a possible  
3 stress situation in sea bream as compared to trout. Furthermore, although glucose levels  
4 were also high the recovery to baseline values was more rapid in this species.

5 In conclusion, the dietary amino acid profile did not modify insulin levels, but  
6 within a profile the decrease in the IAA/DAA ratio induced a lower stimulation of  
7 postprandial insulin in both species, thus suggesting a  $\beta$ -cell sensitivity to small changes  
8 in dietary amino acid proportions. These changes in postprandial insulin secretion did  
9 not affect growth, but appear to contribute to an optimal nutritional and growth process.  
10 Although the amino acid profile modulates postprandial glucagon secretion, individual  
11 amino acids probably have different tropic activities depending on the fish species, thus  
12 illustrating the complexity of glucagon secretion control. A greater emphasis must now  
13 be placed on combined insulin and glucagon responses to diet so as to determine the  
14 metabolic hormonal status that promotes optimal food use and growth.

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25 333038).

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1 **Table legends**

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3 **Table 1.** Ingredients and analytical composition of the experimental diets for rainbow

4 trout.

5 **Table 2.** Analysed amino acid composition of the four experimental diets for rainbow

6 trout

7 **Table 3.** Ingredients and analytical composition of the experimental diets for gilthead

8 sea bream.

9 **Table 4.** Growth performance, feed efficiency parameters and insulin and IGF-I binding

10 parameters in skeletal muscle of rainbow trout fed the experimental diets for

11 12 weeks.

12 **Table 5.** Growth performance, feed efficiency parameters and insulin and IGF-I

13 binding parameters in skeletal muscle of gilthead sea bream fed the

14 experimental diets for 12 weeks.

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3 **Figure legends**

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5 **Figure 1.** Plasma glucose, insulin and glucagon levels of rainbow trout (A, C, E) and  
6 gilthead sea bream (B, D, F). Different letters indicate significant differences  
7 between diet groups for the same time sampling (6 or 24 h) at  $p < 0.05$ . Asterisk  
8 indicates significant differences between sampling time for each diet at  
9  $p < 0.05$ .

10 **Figure 2.** Effect of force feeding on plasma glucose, insulin and glucagon levels of  
11 rainbow trout (A, C, E) and gilthead sea bream (B, D, F). Different letters  
12 indicate significant differences between sampling times (1, 3, 6, 12 and 24 h)  
13 Asterisk indicates significant differences between diets for the same sampling  
14 time of force feeding at  $p < 0.05$ .

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2 **Table 1.-**

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	TM1	TM2	TWB1	TWB2
<i>Ingredients (g/Kg)</i>				
Fish meal (CP 70%)	389,9	316,4	460	363,3
Wheat gluten	71,4	0	50	100
Extruded whole wheat	135,7	71,8	200	200
Extruded peas (Aquatex)	215,1	56,8	166,3	158,5
Soybean meal (CP 42%)	25,3	331,3	0	0
Fish oil	101,6	109,5	93,7	104,1
Binder	10	10	10	10
Mineral premix <sup>a</sup>	10	10	10	10
Vitamin premix <sup>a</sup>	10	10	10	10
CaHPO <sub>4</sub> ·2H <sub>2</sub> O	10,9	16,1	0	0
IAA mix	20,1	18,2	0	0
L-Glu	0	50	0	44,2
<i>Analytical composition (g/Kg)</i>				
Dry matter	922	905	935	921
Protein	451	463	437	441
Lipid	156	164	157	158
NEF <sup>b</sup>	245	200	276	267
Energy (kJ/Kg DM)	224	221	222	224
IAA <sup>c</sup>	209	209	211	179
DAA <sup>d</sup>	202	255	209	256
IAA/DAA	<b>1,03</b>	<b>0,82</b>	<b>1,01</b>	<b>0,70</b>

<sup>a</sup> Mineral and vitamin premix (NRC, 1993).

<sup>b</sup> Nitrogen free extract.

<sup>c</sup> Indispensable amino acids.

<sup>d</sup> Dispensable amino acids.

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1 **Table 2**

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<b>Amino acids (g/Kg DM)</b>	<b>TM1</b>	<b>TM2</b>	<b>TWB1</b>	<b>TWB2</b>
ARGININE	26,0	25,8	27,0	22,8
CYSTEINE	4,9	4,7	4,9	5,0
HISTIDINE	11,0	11,2	10,1	8,6
ISOLEUCINE	19,8	18,5	17,2	14,3
LEUCINE	30,5	29,6	33,3	29,2
LYSINE	32,1	35,4	31,4	24,3
METHIONINE	10,1	10,2	10,4	8,7
PHENYLALANINE	17,1	16,3	18,4	16,6
THREONINE	18,1	18,9	18,1	15,3
TRYPTOPHANE	4,3	5,3	4,5	4,1
TYROSINE	13,2	13,0	14,5	12,7
VALINE	21,9	19,7	21,4	17,8
Alanine	22,1	21,8	24,8	20,5
Aspartic acid	34,0	39,7	37,9	30,9
Glutamic acid	80,9	132,7	76,4	139,5
Glycine	23,7	22,9	26,6	22,3
Proline	22,5	18,7	23,6	24,6
Serine	18,9	19,4	19,7	18,2

Indispensable amino acids (in upper case letters).

Dispensable amino acids (in lower case letters).

1 **Table 3.-**

	<b>SBM1</b>	<b>SBM2</b>	<b>SBWB1</b>	<b>SBWB2</b>
<i>Ingredients (g/Kg)</i>				
Fish meal (CP 70%)	499,9	478,1	529,3	484,1
CPSPG <sup>a</sup>	50	0	0	0
Wheat gluten	0	0	100	100
Extruded whole wheat	0	56,1	147,9	138,5
Extruded peas (Aquatex)	199,2	0	100	100
Soybean meal (CP 42%)	121,9	300	0	0
Fish oil	83,6	94,5	89,2	94,3
Binder	10	10	10	10
Mineral premix <sup>b</sup>	10	10	10	10
Vitamin premix <sup>b</sup>	10	10	10	10
IAA mix	15,3	16,7	3,5	0
L-Glu	0	24,6	0	53,1
<i>Analytical composition (g/Kg)</i>				
Dry matter	921	937	893	927
Protein	527	530	521	509
Lipid	157	164	159	152
NEF <sup>c</sup>	154	159	137	196
Energy (kJ/Kg DM)	225	225	225	225
IAA <sup>d</sup>	259	264	261	225
DAA <sup>e</sup>	228	256	263	280
IAA/DAA	<b>1,13</b>	<b>1,03</b>	<b>0,99</b>	<b>0,80</b>

<sup>a</sup> Fish soluble protein concentrate from Soppopêche (Boulogne sur Mer, France).

<sup>b</sup> Mineral and vitamin premix (NRC, 1993).

<sup>c</sup> Nitrogen free extract

<sup>d</sup> Indispensable amino acids.

<sup>e</sup> Dispensable amino acids.

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1 **Table 4.-**

	TM1	TM2	TWB1	TWB2
IBW (g)	14,1 ± 0,0	14,1 ± 0,0	14,1 ± 0,1	14,1 ± 0,1
FBW (g)	110,0 ± 2,0	100,1 ± 3,9	110,1 ± 8,1	103,6 ± 2,5
HSI (%)	1,9 ± 0,2 <sup>a</sup>	1,4 ± 0,1 <sup>b</sup>	1,9 ± 0,1 <sup>a</sup>	1,8 ± 0,1 <sup>ab</sup>
SGR	2,5 ± 0,0	2,4 ± 0,1	2,5 ± 0,1	2,5 ± 0,0
FGR	1,0 ± 0,0 <sup>a</sup>	0,9 ± 0,0 <sup>b</sup>	1,0 ± 0,1 <sup>a</sup>	1,0 ± 0,0 <sup>a</sup>
PER	2,2 ± 0,0 <sup>a</sup>	2,0 ± 0,1 <sup>b</sup>	2,3 ± 0,1 <sup>a</sup>	2,3 ± 0,1 <sup>a</sup>
Kd INS	0,34 ± 0,1	0,11 ± 0,02	0,12 ± 0,07	0,15 ± 0,09
Ro INS	59,1 ± 17,6	25,3 ± 2,5	22,3 ± 10,1	38,8 ± 5,5
% Bsp INS	1,2 ± 0,34	1,23 ± 0,14	1,93 ± 0,2	2,27 ± 0,5
Kd IGF-1	0,24 ± 0,08	0,06 ± 0,02	0,1 ± 0,04	0,05 ± 0,02
Ro IGF-1	72,0 ± 4,6 <sup>a</sup>	29,9 ± 10,7 <sup>b</sup>	32,0 ± 12,8 <sup>ab</sup>	41,5 ± 5,5 <sup>b</sup>
% Bsp IGF-1	2,8 ± 0,9	2,5 ± 0,6	3,3 ± 0,8	3,5 ± 1,2

IBW: initial body mass; FBW: final body mass; HIS: hepatosomatic index; SGR: specific growth rate ( $[100 \ln(\text{final body weight}) - \ln(\text{initial body weight})]/\text{days}$ ); FGR: feed gain ratio (dry feed intake/wet weight gain); PER: Protein efficiency ratio (wet weight gain/protein intake) (n=15). Values of number of receptor (Ro) are expressed in fmol of receptors per mg of eluted protein, specific binding (%Bsp) in percentage per 20µg of eluted protein and dissociation constant (Kd) in nM for insulin (INS) and IGF-1(n=8). Values are means ± SE. Different superscript letters indicate significant differences between groups, for each parameter studied at  $p < 0,05$ .

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1 **Table 5.-**

	<b>SBM1</b>	<b>SBM2</b>	<b>SBWB1</b>	<b>SBWB2</b>
IBW (g)	14,7 ± 0,2	14,7 ± 0,0	14,6 ± 0,1	14,9 ± 0,1
FBW (g)	74,9 ± 0,7	70,9 ± 1,4	72,9 ± 1,4	70,9 ± 1,2
HSI (%)	1,36 ± 0,0 <sup>bc</sup>	1,2 ± 0,1 <sup>c</sup>	1,54 ± 0,0 <sup>a</sup>	1,5 ± 0,1 <sup>ab</sup>
SGR	2,0 ± 0,0	1,9 ± 0,0	2,0 ± 0,0	1,9 ± 0,0
FGR	1,2 ± 0,0 <sup>a</sup>	1,3 ± 0,1 <sup>ab</sup>	1,2 ± 0,0 <sup>ab</sup>	1,4 ± 0,0 <sup>b</sup>
PER	1,6 ± 0,0	1,5 ± 0,1	1,6 ± 0,0	1,5 ± 0,1
Kd INS	0,08 ± 0,03	0,19 ± 0,07	0,11 ± 0,03	0,38 ± 0,1
Ro INS	34,8 ± 6,8	101,1 ± 32,9	96,2 ± 34,6	124,5 ± 36,1
% Bsp INS	3,1 ± 0,2	4,3 ± 0,5	6,1 ± 0,0	3,0 ± 0,8
Kd IGF-1	0,07 ± 0,01	0,01 ± 0,0	0,03 ± 0,01	0,07 ± 0,02
Ro IGF-1	115,3 ± 12,5	49,0 ± 9,3	54,6 ± 7,3	138,0 ± 34,3
% Bsp IGF-1	11,3 ± 1,4	9,6 ± 1,3	12,6 ± 1,6	13,6 ± 2,2

IBW: initial body mass; FBW: final body mass; HIS: hepatosomatic index; SGR: specific growth rate ([100 ln (final body weight)-ln (initial body weight)]/days); FGR: feed gain ratio (dry feed intake/wet weight gain); PER: Protein efficiency ratio (wet weight gain/protein intake) (n=15). Values of number of receptor (Ro) are expressed in fmol of receptors per mg of eluted protein, specific binding (%Bsp) in percentage per 20µg of eluted protein and dissociation constant (Kd) in nM for insulin (INS) and IGF-1(n=8). Values are means ± SE. Different superscript letters indicate significant differences between groups, for each parameter studied at  $p < 0,05$ .

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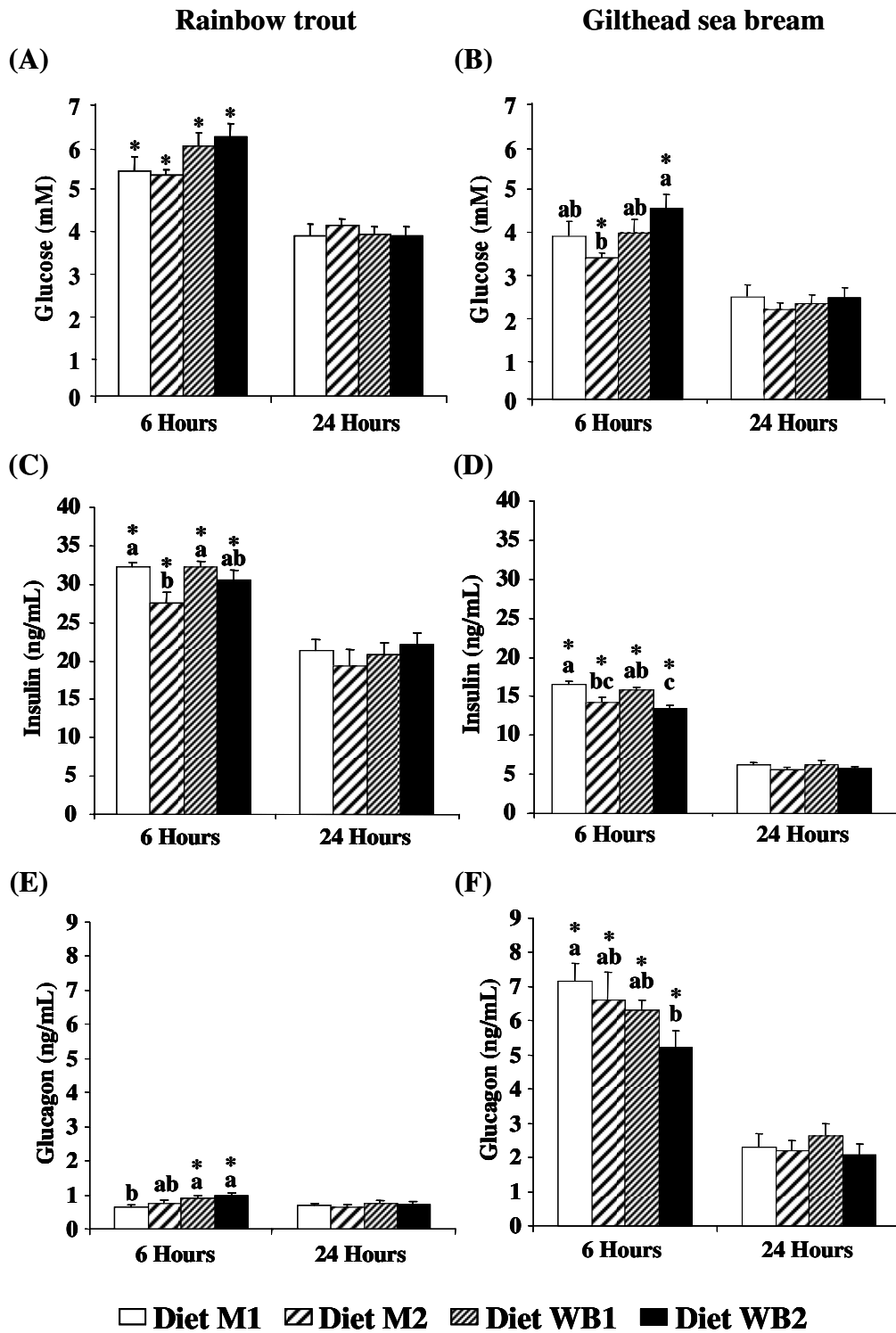
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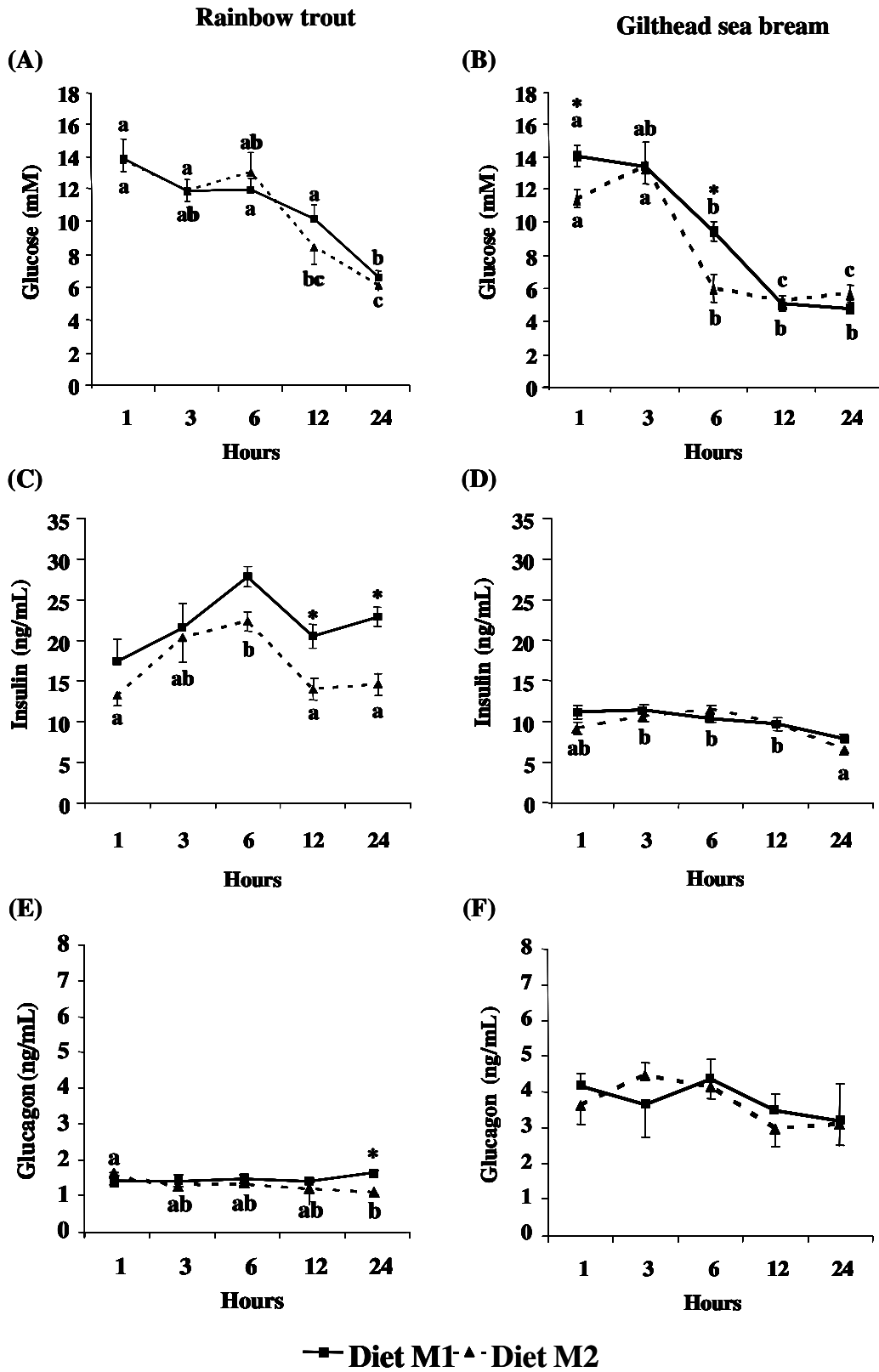


1 Figure 2.-

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## 1   **References**

- 2   1. Baños, N., Planas, J. V., Gutiérrez, J., Navarro, I. (1999) Regulation of plasma  
3    insulin-like growth factor-I levels in brown trout (*Salmo trutta*). *Comp. Biochem.*  
4    *Physiol. C*, **124**, 33-40.
- 5   2. Andoh T. (2007). Amino acids are more important insulinotropins than glucose in a  
6    teleost fish, barfin flounder (*Verasper moseri*). *Gen Comp Endocrinol.* 151:308-317.
- 7   3. Braddford MM (1976) A rapid and sensitive method for quantitation of microgram  
8    quantities of protein utilizing the principle of protein-dye binding. *Analytical*  
9    *biochemistry* 72 248-252.
- 10  4. Burel, C., Boujard, T., Kaushik, S.J., Boeuf, G., Van Den Geyten, S., Mol, K.A.,  
11  Kühn, E.R., Quinsac, A., Krouti, M., Ribaillier, D. (2000) Potential of plant-protein  
12  sources as fish meal substitutes in diets for turbot (*Psetta maxima*): growth, nutrient  
13  utilization and thyroid status. *Aquaculture*, **188**, 363-382.
- 14  5. Carneiro, N. M., Navarro, I., Gutiérrez, J., Plisetskaya, E. M. (1993) Hepatic  
15  extraction of circulating insulin and glucagon in brown trout (*Salmo trutta fario*)  
16  after glucose and arginine injection. *J. Exp. Zool.*, **267**, 416-422.
- 17  6. Castillo, J., Codina, M., Martínez, M.L., Navarro, I., Gutiérrez, J. (2004) Metabolic  
18  and mitogenic effects of IGF-I and insulin on muscle cells of rainbow trout. *Am. J.*  
19  *Physiol. Regul. Integr. Comp. Physiol.*, **286**, R935-R941.
- 20  7. Duncan, N. J., Auchinachie, N., Robertson, D., Murray, R., Bromage, N. (1998)  
21  Growth, maturation and survival of out-of-season 0+ and 1+ Atlantic salmon (*Salmo*  
22  *salar*) smolts. *Aquaculture*, **168**, 325-339.

- 1 8. Francis, G., Makkar, H., Becker, K. (2001) Antinutritional factors present in plant-  
2 derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**,  
3 197-227.
- 4 9. Gannon, M.C., Nuttall, J.A., Nutall, F.Q. (2002) The metabolic response to ingested  
5 glycine. *Am. J. Clin. Nutr.*, **76**, 1302-1307.
- 6 10. Gomez-Requeni, P., Mingarro, M., Kirchner, S., Calduch-Giner, J. A., Medale, F.,  
7 Corraze, G., Panserat, S., Martin, S. A. M., Houlihan, D. F., Kaushik, S. J., Pérez-  
8 Sanchez, J. (2003) Effects of dietary amino acid profile on growth performance, key  
9 metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream  
10 (*Sparus aurata*). *Aquaculture*, **220**, 749-767.
- 11 11. Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin,  
12 S.A.M., Houlihan, D.F., S. Kaushik, S. J., Pérez-Sánchez, J. (2004) Protein growth  
13 performance, amino acid utilisation and somatotropic axis responsiveness to fish  
14 meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*).  
15 *Aquaculture*, **232**, 493–510.
- 16 12. Gutiérrez, J., Zanuy, S., Carrillo, M., Planas, J. (1984) Daily rhythms of insulin and  
17 glucose plasma levels in sea bass *Dicentrarchus labrax* after experimental feeding.  
18 *Gen. Comp. Endocrinol.*, **55**, 393– 397.
- 19 13. Gutiérrez, J., Fernandez, J., Blasco, J., Gesse, J. M., Planas, J. (1986) Plasma-  
20 glucagon levels in different species of fish. *Gen. Comp. Endocrinol.*, **63**, 328-333.
- 21 14. Hemre, G-I, Mommsen, T.P., and Krogdahl, A. (2001). Carbohydrates in fish  
22 nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture*  
23 *Nutr.* 7: 1-20.

- 1 15. Huggett, A.S., Nixon, D.A., (1957) Use of glucose oxidase, peroxidase, and O-  
2 dianisidine in determination of blood and urinary glucose. *Lancet*, **24**, 73 (6991),  
3 368-70.
- 4 16. Ince, B. W., and Thorpe, A. (1977) Glucose and amino acid-stimulated insulin  
5 release in vivo in European silver eel (*Anguilla anguilla* L). *Gen. Comp.*  
6 *Endocrinol.*, **31**, 249-256.
- 7 17. Inui, Y., and Ishioka, H. (1983) Effects of insulin and glucagon on the incorporation  
8 of <sup>14</sup>C-glycine into the protein of the liver and opercular muscle of the eel in vitro.  
9 *Gen. Comp. Endocrinol.*, **51**, 208-212.
- 10 18. Inui, Y., and Yokote, M. (1977) Effects of glucagon on amino-acid metabolism in  
11 Japanese eels, *Anguilla japonica*. *Gen. Comp. Endocrinol.*, **33**, 167-173.
- 12 19. Kaushik, S. J., Cravedi, J., Lalles, J., Sumpter, J., Fauconneau, B., Laroche, M.  
13 (1995) Partial or total replacement of fish meal by soybean protein on growth,  
14 protein utilization, potential estrogenic or antigenic effects, cholesterolemia and  
15 flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **133**, 257-274.
- 16 20. Kaushik, S. J., Coves, D., Dutto, G., Blanc, D. (2004) Almost total replacement of  
17 fish meal by plant protein sources in the diet of a marine teleost, the European  
18 seabass, *Dicentrarchus labrax*. *Aquaculture*, **230**, 391-404.
- 19 21. Kirchner, S., Kaushik, S. J., Panserat, S. (2003) Effect of partial substitution of  
20 dietary protein by a single gluconeogenic dispensable amino acid on hepatic glucose  
21 metabolism in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A.* ,  
22 **134**, 337-347.

- 1 22. Lall, S. P., Kaushik, S. J., Bail, P. Y. I., Keith, R., Anderson, J. S., Plisetskaya, E.  
2 (1994) Quantitative arginine requirement of Atlantic salmon (*Salmo salar*) reared in  
3 sea water. *Aquaculture*, **124**, 13-25.
- 4 23. Larsen, D.A., Beckman, B.R., Dickhoff, W.W. (2001) The effect of low temperature  
5 and fasting during winter on metabolic stores and endocrine physiology (Insulin,  
6 insulin growth factor-I, and thyroxine) of coho salmon, *Oncorhynchus kisutch*. *Gen.*  
7 *Comp. Endocrinol*, **123**, 308-323.
- 8 24. LeRoith D, Werner H, Beitner-Johnson D, and Roberts CT. (1995) Molecular and  
9 cellular aspects of the insulin-like growth factor -I receptor. *Endocr. Rev.*, **16**, 143–  
10 163.
- 11 25. Matty, A., and Lone, K. P. (1985) The hormonal control metabolism and feeding, In  
12 Fish Energetics (New Perspectives), pp. 185-209.
- 13 26. Mommsen, T. P., and Plisetskaya, E. M. (1991) Insulin in fishes and agnathans -  
14 history, structure, and metabolic-regulation. *Rev. Aq. Sci.*, **4**, 225-259.
- 15 27. Mommsen, T.P. (2000). Glucagon-like peptides in fishes: the liver and beyond.  
16 *Amer. Zool.* **40**: 259-268.
- 17 28. Mommsen, T.P. and Busby, E.R. (2006). Glucagon and friends. In “Fish  
18 Endocrinology” Vol 1, Eds. M. Reinecke, G. Zaccane, B.G. Kapoor. Science  
19 Publishers. Pp:223-256.
- 20 29. Moon, T., W. (1998). Glucagon: from hepatic binding to metabolism in teleost fish.  
21 *Comp. Biochem. and Physiol.* **121**: 27-34.

- 1 30. Moon T.W., and Foster, G.D. (1995). Tissue carbohydrate metabolism  
2 gluconeogenesis and hormonal environmental influences. In : Biochemistry and  
3 molecular biology of fishes (P.W. Hochachka and T.P. Mommsen eds.), pp.65-100.  
4 Elsevier , Amsterdam.
- 5 31. Navarro, I., Gutiérrez, J., and Planas J. (1992) Changes in plasma glucagons, insulin  
6 and tissue metabolites associated with prolonged fasting in brown trout (*Salmo*  
7 *trutta fario*) during two different seasons of the year. *Comp. Biochem Physiol. A*,  
8 **102**, 401-407.
- 9 32. Navarro, I., Carneiro, M. N., Párrizas, M., Maestro, J. L., Planas, J., Gutiérrez, J.  
10 (1993) Post-Feeding levels of insulin and glucagon in trout (*Salmo trutta fario*).  
11 *Comp. Biochem. Physiol. A*, **104**, 389-393.
- 12 33. Navarro I, Leibush B, Moon TW, Plisetskaya EM, Baños N, Mendez E, Planas JV,  
13 Gutierrez J. (1999) Insulin, insulin-like growth factor-I (IGF-I) and glucagon: the  
14 evolution of their receptors. *Comp. Biochem. Physiol. B*, **122**, 137-53.
- 15 34. Navarro, I., Gutiérrez, J., Planas, J. (1995) Estimates of fish glucagon by  
16 heterologous radioimmunoassay: antibody selection and cross-reactivities. *Comp.*  
17 *Biochem. Physiol. C*, **110**, 313–319.
- 18 35. Navarro, I., Rojas, P., Capilla, E., Albalat, A., Castillo, J., Montserrat, N., Codina,  
19 M., Gutiérrez, J. (2002) Insights into insulin and glucagon responses in fish. *Fish*  
20 *Physiology and Biochemistry* 27, 205-216. Special Issue: “Fish growth and  
21 metabolism. environmental, nutritional and hormonal regulation”. E. M. Plisetkaya,  
22 Ed., (published 2004).

- 1 36. National Research Council (NRC), (1993) Nutrient requirements of fish. National  
2 Academy Press, Washington, DC. 124 pp..
- 3 37. Novoa, M. D., Capilla, E., Rojas, P., Baro, J., Gutiérrez, J., Navarro, I. (2004)  
4 Glucagon and insulin response to dietary carbohydrate in rainbow trout  
5 (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.*, **139**, 48-54.
- 6 38. Nutall, F.Q., Schweim, K.J., Gannon, M.C. (2006) Effect of orally administered  
7 phenylalanine with and without glucose on insulin, glucagon and glucose  
8 concentrations. *Horm. Metabol. Res.*, **38**, 518-523.
- 9 39. Papatryphon, E., Capilla, E., Navarro, I., Soares, J. H. (2001) Early insulin and  
10 glucagon response associated with food intake in a teleost, the striped bass (*Morone*  
11 *saxatilis*). *Fish Physiol. Biochem.*, **24**, 31-39.
- 12 40. Pek, S., Fajans, S.S., Floyd, J.C., Jr., Knopf, R.F., Conn, J.W. (1969) Effects upon  
13 plasma glucagon on infused and ingested amino acids and of protein meals in man.  
14 *Diabetes*, **18**, Suppl. 1, 328.
- 15 41. Pérez, J., Zanuy, S., Carrillo, M. (1988) Effects of diet and feeding time on daily  
16 variations in plasma-insulin, hepatic camp and other metabolites in a teleost fish,  
17 *Dicentrarchus labrax* L. *Fish Physiol. Biochem.* **5**, 191-197.
- 18 42. Pérez-Sanchez, J., and Le Bail, P. Y. (1999) Growth hormone axis as marker of  
19 nutritional status and growth performance in fish. *Aquaculture*, **177**, 117-128.
- 20 43. Peter, R. E., and Marchant, T. A. (1995) The endocrinology of growth in carp and  
21 related species. *Aquaculture*, **129**, 299-321.



- 1 44. Plisetskaya, E. M. (1989) Physiology of fish endocrine pancreas. *Fish Physiol.*  
2 *Biochem.*, **7**, 39-48.
- 3 45. Plisetskaya E.M. and Mommsen, T.P. (1996). Glucagon and glucagon-like peptides  
4 in fishes. *Int Rev. Cytol.* **168**: 187-256.
- 5 46. Plisetskaya, E. M., Buchelli-Narvaez, L. I., Hardy, R. W., Dickhoff, W. W. (1991)  
6 Effects of injected and dietary arginine on plasma-insulin levels and growth of  
7 Pacific salmon and rainbow trout. *Comp. Biochem. Physiol. A*, **98**, 165-170.
- 8 47. Rocha, D.M., Faloona, G.R., Unger, R.H. (1972) Glucagon-stimulating activity of  
9 20 amino acids in dogs. *J. Clin. Invest.*, **51**, 234-2351.
- 10 48. Sala-Rabanal, M., Sánchez, J., Ibarz, A., Fernández-Borràs, J., Blasco, J., Gallardo,  
11 M.A. (2003) Effects of low temperatures and fasting on hematology and plasma  
12 composition of gilthead sea bream (*Sparus aurata*). *Fish Physiol. Biochem.*,  
13 **29**, 105-115.
- 14 49. Sauvant D., Perez J.-M., Tran G. (2004) Tables of composition and nutritive value  
15 of feed materials : Pigs, poultry, cattle, sheep, goats, rabbits, horses, fish., INRA  
16 Editions Versailles. 304p.
- 17 50. Sundby, A., Eliassen, K. A., Blom, A. K., Asgard, T. (1991) Plasma-Insulin,  
18 glucagon, glucagon-like peptide and glucose levels in response to feeding,  
19 starvation and life long restricted feed ration in salmonids. *Fish Physiol. Biochem.*,  
20 **9**, 253-259.

- 1 51. Sundby, A., Eliassen, K., Refstie, T., Plisetskaya, E.M. (1991). Plasma levels of  
2 insulin, glucagon and glucagon-like peptide in salmonids of different weights. *Fish*  
3 *Physiol. Biochem.* 9, 223-230.
- 4 52. Vega-Rubín de Celis S., Rojas, P., Gomez-Requeni, P., Albalat, A., Gutiérrez, J.,  
5 Medale, F., Kaushik, S. J., Navarro, I., Pérez-Sanchez, J. (2004) Nutritional  
6 assessment of somatolactin function in gilthead sea bream (*Sparus aurata*):  
7 concurrent changes in somatotropic axis and pancreatic hormones. *Comp. Biochem.*  
8 *Physiol. A*, **138**, 533-542.
- 9 53. Vielma, J., Mäkinen, T., Ekholm, P., Koskela, J., (2000) Influence of dietary soy  
10 and phytase levels on performance and body composition of large rainbow trout  
11 (*Oncorhynchus mykiss*). *Aquaculture*, **183**, 349-362.
- 12 54. Watanabe, T., Verakunpiriya, V., Watanabe, K., Viswanath, K., Satoh, S. (1998)  
13 Feeding of rainbow trout with non-fish meal diets. *Fish. Sci.*, **63**,(2):258-266, 1997.
- 14 55. Wood, A.W., Duan, C., Bern, H.A. (2005) Insulin-like growth factor signalling in  
15 fish. *Int. Rev. Cytol.*, **243**, 215-285.

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